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Original article

Synthesis of bioactive polyheterocyclic ring systems as 5α-reductase inhibitors

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ABSTRACT

Simple synthetic strategies for the hitherto unreported [1,2,4]triazolo[4,3-*a*]pyrido[4,3-*d*]pyrimidines **8** and [1,2,4]triazolo[4′,3′:1,2]pyrimido[4,5-*b*][1,6]naphthyridine-5-one **15** are described based on reaction of thione **3** and **12** with hydrazonoyl chloride **1a**–**h**, respectively. The structures of products **8** and **15** were confirmed by spectroscopic and X-ray crystallographic analyses. Also, the mechanism of such reactions was discussed. In addition, reaction of compound **12** with bromoacetic acid and hydrazine hydrate was investigated. Compounds were screened against 5α-reductase and showed activities with good LD_{50} and LD_{90} for all compounds.

1. Introduction

Heterocycles bearing pyrimidine and pyridopyrimidine moieties are reported to show a broad spectrum of pharmacological properties such as antimicrobial [1–5], central nervous system (CNS), analgesic, anti-inflammatory [6–9], and anti-HIV [10]. In addition, [1,2,4]triazolo[4,3-*a*]pyrimidines are pharmacological scaffold that represent a wide range of biological activities such as antitumor [11], and anti-inflammatory [12]. Based on these findings, we report herein the synthesis of [1,2,4]triazolo[4,3-*a*]pyrido [4,3-*d*]pyrimidines and [1,2,4]triazolo[4′,3′:1,2]pyrimido [4,5-*b*] [1,6]naphthyridine-5-ones that have not been reported hitherto. Also, we study the biological activity of the newly synthesized compounds against 5α-reductase activity.

2. Chemistry

1-Ethyl-4-piperidinone **1** was reacted with 4-methylbenzaldehyde in ethanol and potassium hydroxide to yield 1-ethyl-3,5-bis(4-methylphenylmethylene)-piperidin-4-one **2** (Scheme 1). The structure of the isolated product **2** was verified by elemental analyses and spectroscopic methods (IR, ^{1}H NMR and MS) (see Experimental). Compound **2** was then reacted with thiourea in ethanol in presence of potassium hydroxide to yield 6-ethyl-8-(4-methylphenylmethylene)-4-(4-methylphenyl)-3,4,5,6,7,8-hexahydropyrido [4,3-*d*]pyrimidine-2(1*H*)-thione **3** (cf. Scheme 1). Also, reaction of **3** with methyl iodide in dimethylformamide in presence of anhydrous potassium carbonate afforded 6-ethyl-8-(4-methylphenylmethylene)-2-(methylthio)-4-(4-methylphenyl)-3,4,5,6,7,8-hexahydropyrido[4,3-*d*]pyrimidine **4** (cf. Scheme 1). The structure of products **3** and **4** were evidenced based on mass, IR and ^{1}H NMR spectral data (see Experimental).

Reaction of **3** with the appropriate hydrazonoyl chlorides **5** in dioxane in the presence of triethylamine under reflux was found to give in each case one isolable product (TLC) that was identified to be [1,2,4]triazolo[4,3-*a*]pyrido[4,3-*d*]pyrimidines **8** rather than its isomeric structure [1,2,4]triazolo[4,3-*a*]pyrido[3,4-*e*]pyrimidines **9** (Scheme 2). The direct formation of products **8** from the reaction of compound **3** with hydrazonoyl chloride **5** indicates that the intermediate thiohydrazonate esters **6** underwent Smiles rearrangement [13] to give the corresponding thiohydrazides **7**, which *in situ* underwent cyclization with concurrent elimination of hydrogen sulfide gas to give **8** as end products (cf. Scheme 2). All attempts to isolate the intermediates **6** and **7** were failed since they were consumed as soon as they were formed under the employed reaction conditions. Alternatively, the formation of **8** from 2-methylthio derivative **4** and hydrazonoyl chloride **5** can be accomplished *via*

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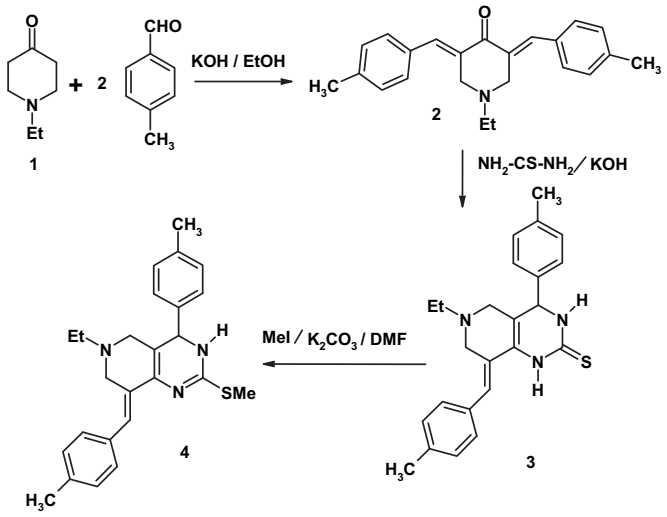
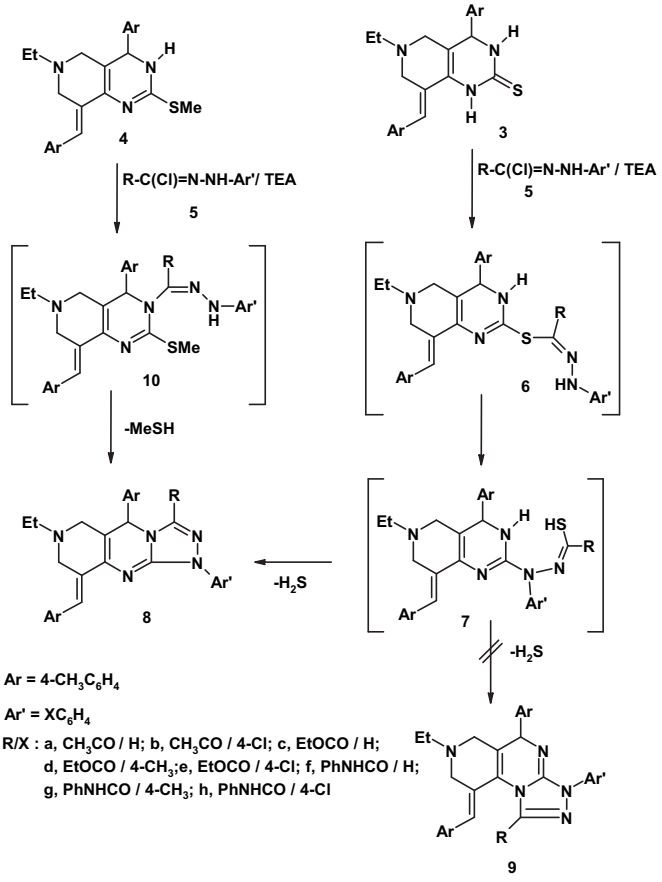


Scheme 1. Synthesis of compounds **3** and **4**.

cyclization of the intermediate amidrazone **10** with concurrent elimination of methanethiol to give **8** as end products (cf. Scheme 2). The structure of products **8** cannot be distinguished from the discarded products **9** by Ms, IR, ^{1}H NMR or elemental analyses. So, we turned to X-ray crystallographic analysis as an exclusive evidence for elucidating the structure of products **8**. The ORTEP drawing of **8b** isolated from the reaction of **3** with **5b**, taken as a typical example of the series prepared, is shown in Fig. 1 with selected bond lengths and bond angles depicted in Table 1 [14].



Scheme 2. Synthesis of compound **8**.

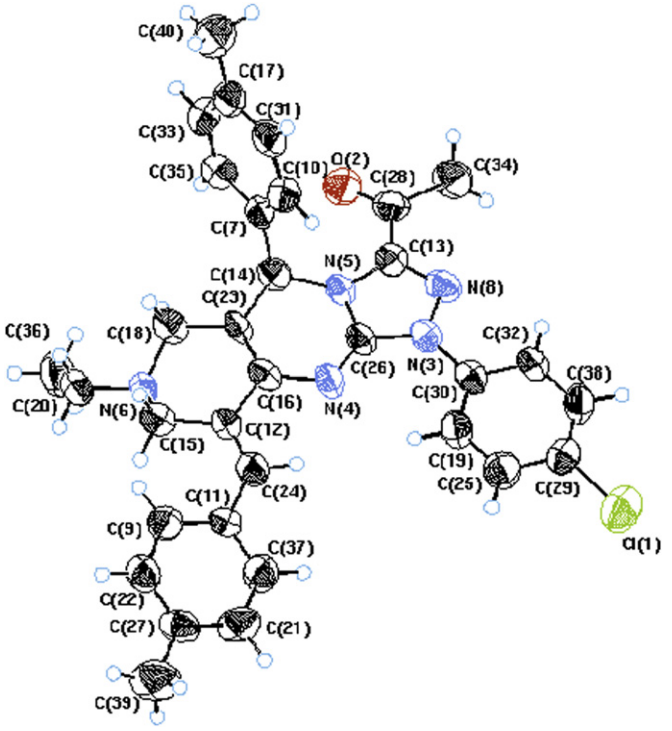


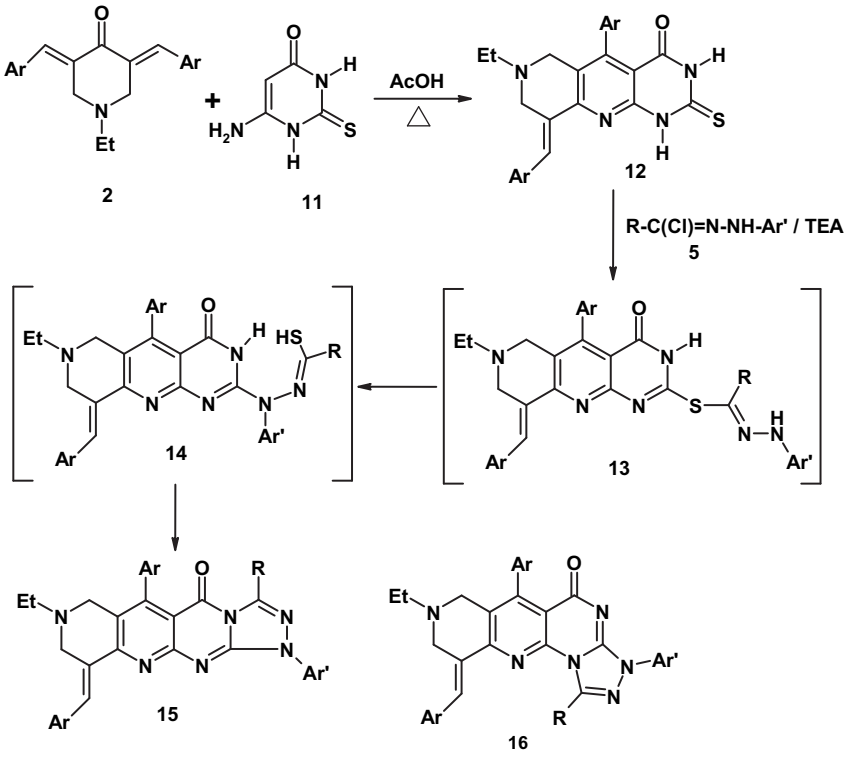
Fig. 1. The ORTEP drawing of **8b**.

Reaction of arylidene **2** with 6-amino-2-thioxo-pyrimidine-4(3*H*)-one **11** in acetic acid under reflux afforded 7-ethyl-9-(4-methylphenylmethylene)-5-(4-methylphenyl)-2-thioxo-1,2,6,7,8,9-hexahydro-pyrimido[4,5-*b*][1,6]naphthyridine-4(3*H*)-one **12**. The structure of compound **12** was established on the basis of Ms, IR and ^{1}H NMR spectral data (see Experimental).

Reaction of the thione **12** with hydrazonoyl chloride **5a**,**c**,**h** in dioxane in presence of triethylamine afforded in each case a single product. Both spectroscopic data and elemental analyses were consistent with either compound **15** or **16** (Scheme 3). An immediate distinction between these two structures was reached by comparison of the ^{13}C NMR spectral data with those of similar annelated pyrimidinones. Literature reports [15–18] have shown that the chemical shift for the carbonyl carbon in 4-pyrimidinone

Table 1
Selected bond lengths and bond angles in the ORTEP of compound **8b** in the crystal. The crystallographic numbering does not reflect systematic numbering.

Bond length, Å	Bond length, Å	Bond length, Å
C11–C29, 1.742	N4–C26, 1.279	C11–C24, 1.462
O2–C28, 1.211	N5–C14, 1.474	C12–C15, 1.505
N3–N5, 2.171	N6–C15, 1.458	C12–C16, 1.473
N3–N8, 1.370	N6–C18, 1.455	C20–C36, 1.501
N3–C26, 1.393	C7–C10, 1.381	C22–C27, 1.390
N3–C30, 1.416	C7–C14, 1.519	N6–H15A, 1.988
N4–C16, 1.424	N8–C13, 1.302	N6–H20A, 2.007
Angle (ω)	**Angle (ω)**	**Angle (ω)**
C24–C12–H15A, 119.1	H31–C17–H40A, 121.6	C9–C22–H9, 23.9
C24–C12–H15B, 99.9	H31–C17–H40B, 82.1	C13–N5–H14, 111.3
C7–C14–H14, 107.8	H33–C17–H40A, 71.5	C14–N5–H14, 26.56
N6–C15–H15A, 108.8	H40–C17–H40B, 44.66	C26–N5–H14, 135.1
C12–C15–H15A, 108.3	C36–C20–H20A, 108.8	C15–N6–H15A, 27.20
C31–C17–H31, 24.6	C36–C20–H20B, 107.3	C15–N6–H15B, 27.24
C33–C17–H31, 143	C36–C20–H36C, 26.45	C18–N6–H15A, 95.1
C40–C17–H31, 95.7	C27–C21–H21, 118.3	C7–C10–H10, 118.7
C40–C17–H33, 97.4	C27–C21–H37, 145.3	C24–C11–H24, 23.67
C40–C17–H40A, 25.9	C37–C21–H21, 120.5	C15–C12–H15A, 26.78



Scheme 3. Synthesis of compound **15**.

derivatives is markedly affected by the nature of the adjacent nitrogen (N3) (pyrrole type in structure **15** and pyridine type as in structure **16**). For example, the ^{13}C NMR spectra of **15a** taken as typical example of the series prepared, revealed the signal of the carbonyl carbon of the pyrimidinone ring residue at δ 162 ppm. Such chemical shift value is similar to that of annelated pyrimidinones of pyrrole type rather than that of the pyridine type. On the basis of this similarity, the isolated products were assigned structure **15** and structure **16** was therefore excluded.

Finally, compound **12** reacted with 2-bromoacetic acid in acetic acid and acetic anhydride in presence of fused sodium acetate to give thiazolo[3′,2′:1,2]pyrimido[4,5-*b*][1,6]naphthyridine-3,5-dione **17**. Treatment of the later product **17** with 3,4-dimethoxy-benzaldehyde afforded 2-(3,4-dimethoxyphenylmethylene)-thiazolo[3′,2′:1,2]pyrimido[4,5-*b*][1,6]naphthyridine-3,5-dione **18**. Compound **18** was also prepared by one step reaction *via* 2-bromoethanoic acid and 3,4-dimethoxy-benzaldehyde under the same reaction conditions (Scheme 4).

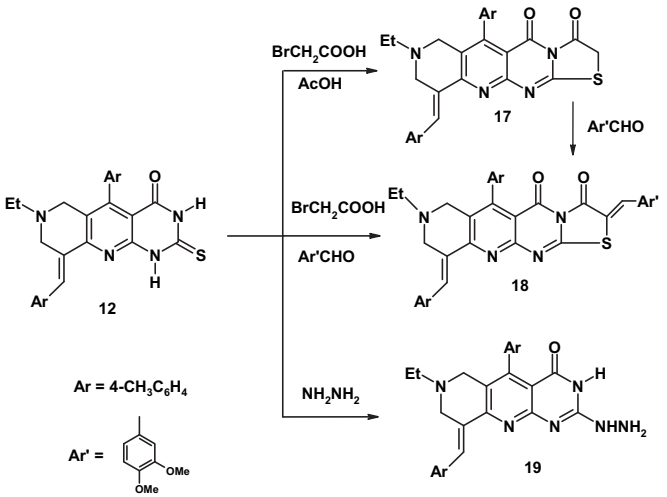
Reaction of compound **12** with hydrazine hydrate led to formation of 2-hydrazino derivatives **19**. The structure of all products **17**–**19** was established on the basis of mass, IR, ^{1}H NMR and elemental analyses.

3. Pharmacology

3.1. Biological assay

Treatment of animals – Animals were obtained from the animal house colony of the National Research Center, Cairo, Egypt. All animals were allowed free access to water and were kept on a constant standard diet. Twenty-three groups, each of 12 male Sprague–Dawley rats in the postnatal third days, were treated subcutaneously with the 5α-reductase inhibitor (tested compound or reference standard). The tested compounds were dissolved in 5% Tween 80 in water. The solvent was used for both standard and negative control group, beginning on the postnatal third day until the age of seven weeks.

Twenty-one groups were used to test the activities, of which one was used as the positive control for anastrozole and another served as the negative control group. After, scarifying blood was



Scheme 4. Reaction of compound **12** with bromoethanoic acid and hydrazine hydrate.

withdrawn for testosterone and dihydrotestosterone (DHT) determination [19]. Moreover, intraprostatic concentrations of testosterone and DHT were determined [20].

The biological experiments were performed according to the official standards.

Radioimmuno assay for testosterone and dihydrotestosterone – Serum testosterone and dihydrotestosterone were measured by radioimmunoassay in serum extracts using specific antisera without prior chromatography. Serum samples of 0.5 mL were extracted with 2 mL of freshly purified peroxide-free diethyl ether by shaking for 60 s on a Vortex mixer. The aqueous phase was frozen at −70 °C, the ether phase containing steroids was transferred to a conical test tube and evaporated in BSA/phosphate buffer (pH = 7.4) containing (1,2,6,7-3*H*)-testosterone or (1,2,6,7-3*H*)-dihydrotestosterone and then specific antisera were added and incubated over a period of 24 h at 4 °C under non-equilibrium conditions. Bound hormone and free hormone were separated by adsorption on dextran-coated charcoal. The activity of each sample was determined in a Beckman-counter (USA) using a commercially available scintillation cocktail (Mini-RIA, Zinsser, Spain). As for other steroid hormones, commercially available KIA-kits, *e.g.*, Biermann GmbH, Germany, can be used. The hormone level in the sample was calculated from a standard curve by means of a computer program (KIA-Calc, LKB, Canada), using appropriate control sera. Steroid levels of rats treated with different doses of 5-reductase inhibitors were compared with vehicle-treated controls (Table 1). The relative potency was calculated by dividing the ED_{50} (dose that causes 50% of pharmacological response in the test) of anastrozole by that of a tested compound.

3.2. Determination of acute toxicity

LD_{50} and LD_{90} were determined by using male albino rats and injecting them with different increasing doses of agents. Doses that killed 50% and 90% of the tested animals, respectively, were calculated according to Austen et al. [21] (Table 2).

3.3. Pharmacological screening

Circulating testosterone and dihydrotestosterone hormone levels or tissue concentrations were measured after administration of 5α-reductase inhibitor radioimmuno assays. All synthesized compounds were tested for their 5α-reductase inhibitor activity *in vivo;* the ED_{50}, LD_{50} and LD_{90} data were determined and are given in Table 2.

Table 2
Evaluation of ED_{50}, LD_{50}, LD_{90} and 5α-reductase inhibitor activities relative to anastrozole.

Compd. No.	ED_{50}[a] (μg kg^{-1})	LD_{50}[b] (μg kg^{-1})	LD_{90}[c] (μg kg^{-1})	Potency relative to Anastrozole
3	1.88	222.23	434.76	0.579787
8d	1.00	224.33	5 14.33	1.09
8g	0.925	23 6.28	417.34	1.178378
8a	0.911	257.23	419.23	1.196487
8b	0.856	3 12.34	735.34	1.273364
15h	0.811	413.34	848.56	1.34402
12	0.795	447.88	937.34	1.371069
17	0.766	516.56	1148.50	1.422977
15c	0.611	823.45	1226.89	1.783961
8e	0.441	712.55	1438.60	2.471655
8f	0.432	6534.45	1950.12	2.523148
Anastrozole	1.09	2.415	3.69	1.00

[a] ED_{50}: Dose caused 50% of pharmacological response in the test.
[b] LD_{50}: Dose killed 50% of the tested animals.
[c] LD_{90}: Dose killed 90% of the tested animals.

All the tested compounds showed 5α-reductase inhibitor activities with good LD_{50} and LD_{90} for all compounds were high enough to provide good therapeutic windows and a soft profile margin. The ascending order of activity is **3**, **8d**, **8g**, **8a**, **8b**, **15h**, **12**, **17**, **15c**, **8e** and **8f**.

4. Conclusion

In conclusion, simple synthetic strategies for the synthesis of tri and tetra heterocyclic ring systems namely [1,2,4]triazolo[4,3-*a*]pyrido[4,3-*d*]pyrimidines **8** and [1,2,4]triazolo[4′,3′:1,2]pyrimido[4,5-*b*][1,6]naphthyridine-5-one **15** were described. The structures of compounds **8** and **15** were confirmed by spectroscopic and X-ray crystallographic analyses. Also, the mechanism of such reactions was discussed. In addition, all synthesized compounds were tested for their 5α-reductase activity *in vivo*. All the tested compounds showed 5α-reductase activities with good LD_{50} and LD_{90} for all compounds were high enough to provide good therapeutic windows and a soft profile margin.

5. Experimental protocol

5.1. Chemistry

All melting points were determined on an electrothermal Gallenkamp apparatus. Solvents were distilled and dried by standard literature procedures prior to use. The IR spectra were measured on a Pye-Unicam SP300 instrument in potassium bromide discs. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) and the chemical shifts were related to that of the solvent DMSO-d_6. The mass spectra were recorded on a GCMS-Q1000-EX Shimadzu and GCMS 5988-A HP spectrometers, the ionizing voltage was 70 eV. Elemental analyses were carried out by the Microanalytical Center of Cairo University, Giza, Egypt. Hydrazonoyl halides **5a**–**h** were prepared following literature methods [22,23].

5.2. Crystallographic analysis

The crystals were mounted on a glass fiber. All measurements were performed on an ENRAF NONIUS FR 590. The data were collected at a temperature of 25 °C using the ω scanning technique to a maximum of a 20 of 22.986°. The structure was solved by direct method using SIR 92 and refined by full-matrix least squares. Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were located geometrically and were refined isotropically.

5.2.1. Crystal data

For compound **8b**: $C_{33}H_{32}ClN_5O$, M = 550.106, monoclinic, a = 12.5470 (6), b = 9.6393 (4), c = 24.1177 (14) Å, v = 2904.3 (2), $\alpha = \gamma = 90.00°$, β = 95.326 (2), space group: $P2_1/c$, Z = 4, D_x = 1.258 Mg m^{-3} reflection 1,0911 measured, θ_{max} = 27.43°. Fig. 1 illustrates the structure as determined. Full data can be obtained on request from the CCDC [14].

5.3. Preparation of 1-ethyl-3,5-bis(4-methylbenzylidene)piperidin-4-one (*2*)

To a mixture of compound **1** (1.27 g, 0.01 mol) and 4-methylbenzaldehyde (2.40 g, 0.02 mol) in ethanol (100 mL) was added potassium hydroxide (1 g) in few drops of water. The mixture was stirred at room temperature for 30 min, the solid formed was collected, washed with ethanol and crystallized from ethanol to give compound **2** as yellow crystals, yield (95%), mp 210 °C ^{1}H NMR ($CDCl_3$) at δ = 0.80 (t, J = 7 Hz, 3H, CH_3), 2.31 (q, J = 7 Hz, 2H, CH_2),

2.53 (s, 6H, $2CH_3$), 3.12–3.40 (4d, 4H, $2CH_2$), 7.61 (s, 2H, benzylic–H), 7.63–7.80 (m, 8H, ArH), IR (KBr, cm^{-1}) 1676. MS *m/z* (%) 332 (M^++1, 14), 331 (M^+, 100), 104 (61), 91 (95), 77 (93). Anal. Calcd. For $C_{23}H_{25}NO$ (331.46): C, 83.34; H, 7.60; N, 4.23. Found C, 83.21; H, 7.36; N, 4.10%.

5.4. Preparation of 6-ethyl-8-(4-methylphenylmethylene)-4-(4-methylphenyl)-3,4,5,6,7,8-hexahydropyrido[4,3-d]pyrimidine-2(1H)-thione (**3**)

To a boiling mixture of compound **2** (3.3 g, 0.01 mol) in ethanol (100 mL) containing potassium hydroxide (1 g) in water (0.5 mL) was added thiourea (0.76 g, 0.01 mol) and the reaction mixture was refluxed for 3 h, allowed to cool and the solid formed was filtered off, dissolved in water and precipitated by HCl. The solid formed was filtered off and crystallized from ethanol to afford compound **3** as white crystals. Yield (85%) mp 260 °C ^{1}H NMR (DMSO-d_6) at $\delta = 0.83$ (t, $J = 7$ Hz, 3H, CH_3), 2.29 (q, $J = 7$ Hz, 2H, CH_2), 2.49 (s, 6H, $2CH_3$), 2.61–3.47 (4d, 4H, $2CH_2$), 4.82 (s, 1H, pyrimidine–H), 7.08 (s, 1H, benzylic–H), 7.17–7.18 (m, 8H, ArH), 9.04 (s, 1H, NH, exchangeable with D_2O), 9.40 (s, 1H, 1NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6) 12.07, 20.69, 20.79, 50.35, 51.68, 51.84, 56.83, 111.83, 122.51, 125.94, 126.17, 126.66, 128.92, 129.03, 129.22, 133.50, 136.30, 137.15, 139.65, 174.01. MS *m/z* (%) 389 (M^+, 100), 287 (20), 104 (45), 91 (90), 77 (72). Anal. Calcd. For $C_{24}H_{27}N_3S$ (389.57): C, 74.00; H, 6.99; N, 10.79. Found C, 73.92; H, 6.84; N, 10.65%.

5.5. Preparation of 6-ethyl-8-(4-methylphenylmethylene)-2-(methylthio)-4-(4-methyphenyl)-3,4,5,6,7,8-hexahydropyrido[4,3-d]pyrimidine (**4**)

To a stirred solution of compound **3** (1.9 g, 5 mmol) in DMF (20 mL) was added anhydrous potassium carbonate (0.70 g, 5 mmol) and methyl iodide (0.71 g, 5 mmol). The reaction mixture was stirred overnight at room temperature then poured onto ice-water. The solid formed was filtered off, washed with water, dried and crystallized from ethanol to give compound **4** as yellow solid. Yield (68%) mp 244 °C ^{1}H NMR (DMSO-d_6) at $\delta = 1.10$ (t, $J = 7$ Hz, 3H, CH_3), 2.25(s, 3H, SCH_3), 2.35 (q, $J = 7$ Hz, 2H, CH_2), 2.41 (s, 6H, $2CH_3$), 2.64–3.46 (m, 4H, $2CH_2$), 4.96 (s, 1H, pyrimidine–H), 7.25–7.50 (m, 9H, ArH + benzylic–H), 8.62 (s, 1H, NH, exchangeable with D_2O). IR (KBr, cm^{-1}) 3424 (NH) MS *m/z* (%) 403 (M^+, 25), 401 (91), 400 (100), 386 (56), 296 (66), 142 (75), 118 (44), 77(40). Anal. Calcd. For $C_{25}H_{29}N_3S$ (403.59): C, 74.40; H, 7.24; N, 10.41. Found C, 74.21; H, 7.06; N, 10.20%.

5.6. Preparation of [1,2,4]triazolo[4,3-a]pyrido[4,3-d]pyrimidines (**8a**–**h**)

Method A: To a mixture of equimolar amount of **3** and the appropriate hydrozonyl chloride **5a**–**h** (2.5 mmol of each) in dioxane (20 mL) was added triethylamine (0.35 mL, 2.5 mmol). The reaction mixture was refluxed till all of the starting materials have disapppeared and hydrogen sulfide gas ceased to evolve (10 h monitored by TLC). The solvent was evaporated and the residue was treated with methanol. The solid that formed was filtered off and crystallized from the appropriate solvent to give compounds **8a**–**h**.

Method B: To a mixture of equimolar amount of **4** and the appropriate hydrozonyl chlorides **5b**,**d**,**f** (2.5 mmol of each) in dioxane (20 mL) was added triethylamine (0.35 mL, 2.5 mmol). The reaction mixture was refluxed till all methanthiol gas ceased to evolve (20 h, monitored by TLC). The solvent was evaporated and the residue was treated with methanol. The solid that formed was filtered off and crystallized from the appropriate solvent to give products identical in all respects (mp, mixed mp and IR) with that were formed from Method A.

5.6.1. 3-Acetyl-1-phenyl-5-(4-methylphenyl)-9-(4-methylphenylmethylene)-7-ethyl-5H-6,7,8,9-tetrahydro[1,2,4]triazolo[4,3-a]pyrido[4,3-d]pyrimidine (**8a**)

Yellow crystal, yield (85%) mp 200–202 °C (Ethanol). IR (KBr, cm^{-1}) 1651 (CO), MS *m/z* (%) 516 (M^++1, 2), 515 (M^+, 8), 445 (20), 251(100), 208 (80), 83 (12). Anal. Calcd. For $C_{33}H_{33}N_5O$(515.66): C, 76.87; H, 6.45; N, 13.58, Found C, 76.59; H, 6.30; N, 13.42%.

5.6.2. 3-Acetyl-7-ethyl-1-(4-chlorophenyl)-5-(4-methylphenyl)-9-(4-methylphenylmethylene)-5H-6,7,8,9-tetrahydro[1,2,4]triazolo[4,3-a]pyrido[4,3-d] pyrimidine (**8b**)

Golden yellow crystal, yield (82%) mp 194–196 °C (Ethanol). ^{1}H NMR (DMSO-d_6) at $\delta = 0.95$ (t, $J = 7$ Hz, 3H, CH_3), 2.29 (s, 6H, $2CH_3$), 2.36 (s, 3H, CH_3), 2.41 (q, $J = 7$ Hz, 2H, CH_2), 2.75–3.76 (4d, 4H, $2CH_2$), 6.22 (s, 1H, pyrimidine–H), 7.25–8.34 (m, 13H, ArH + benzylic–H). IR (KBr, cm^{-1}) 1695 (CO). MS *m/z* (%) 552 ($M^+ + 2$, 8), 551 ($M^+ + 1$, 15), 550 (M^+, 56), 548 (100), 129 (25), 105 (34), 91 (37). Anal. Calcd. For $C_{33}H_{32}ClN_5O$ (550.11): C, 72.05; H, 5.86; N, 12.73. Found C, 71.96; H, 5.63; N, 12.54%.

5.6.3. 3-Ethoxycarbonyl-7-ethyl-1-phenyl-5-(4-methylphenyl)-9-(4-methylphenylmethylene)-5H-6,7,8,9-tetrahydro[1,2,4]triazolo[4,3-a]pyrido[4,3-d]pyrimidine (**8c**)

Orange crystal, yield (85%) mp 164–166 °C (Ethanol). ^{1}H NMR (DMSO-d_6) at $\delta = 1.07$ (t, $J = 7$ Hz, 3H, CH_3), 1.20 (t, 3H, CH_3), 2.24 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.56 (q, $J = 7$ Hz, 2H, CH_2), 3.19–3.38 (4d, 4H, $2CH_2$), 4.21 (q, 2H, CH_2), 5.85 (s, 1H, pyrimidine–H), 7.02–8.01 (m, 14H, ArH + benzylic–H). IR (KBr, cm^{-1}) 1731 (CO). MS *m/z* (%) 546 ($M^+ + 1$, 2), 545 (M^+, 6), 445 (21), 184 (17), 118 (27), 91 (100). Anal. Calcd. For $C_{34}H_{35}N_5O_2$ (545.69): C, 74.84; H, 6.47; N, 12.83. Found C, 74.60; H, 6.35; N, 12.56%.

5.6.4. 3-Ethoxycarbonyl-7-ethyl-1-(4-methylphenyl)-5-(4-methylphenyl)-9-(4-methylphenylmethylene)-5H-6,7,8,9-tetrahydro[1,2,4]triazolo[4,3-a]pyrido[4,3-d] pyrimidine (**8d**)

Yellow crystal, yield (75%), mp 220–222 °C (Ethanol). ^{1}H NMR (DMSO-d_6) at $\delta = 0.83$ (t, $J = 7$ Hz, 3H, CH_3), 1.18 (t, 3H, CH_3), 2.22 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 3.13 (q, $J = 7$ Hz, 2H, CH_2), 3.29–3.53 (4d, 4H, $2CH_2$), 4.22 (q, 2H, CH_2), 6.17 (s, 1H, pyrimidine–H), 7.13–8.11 (m, 13H, ArH + benzylic–H). IR (KBr, cm^{-1}) 1725 (CO). Anal. Calcd. For $C_{35}H_{37}N_5O_2$ (559.72): C, 75.11; H, 6.66; N, 12.51. Found C, 75.34; H, 6.29; N, 12.61%.

5.6.5. 3-Ethoxycarbomyl-7-ethyl-1-(4-chlorophenyl)-9-(4-methylphenylmethylene)-5H-6,7,8,9-tetrahydro[1,2,4]triazolo[4,3-a]pyrido[4,3-d]pyrimidine (**8e**)

Yellow solid, yield (78%) mp 228 °C (Ethanol). ^{1}H NMR (DMSO-d_6) at $\delta = 1.12$ (t, $J = 7$ Hz, 3H, CH_3), 1.28 (t, $J = 7$ Hz, 3H, CH_3), 2.24 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.55–3.72 (m, 6H, $3CH_2$), 4.01 (q, $J = 7$ Hz, 2H, CH_2), 5.94 (s, 1H, pyrimidine–H), 7.02–8.00 (m, 13H, ArH + benzylic–H). IR (KBr, cm^{-1}) 1694 (CO). MS *m/z* (%) 581 ($M^+ + 1$, 10), 580 (M^+, 30), 576 (25), 564 (35), 430 (66), 111 (100), 90 (70). Anal. Calcd. For $C_{34}H_{34}ClN_5O_2$ (580.14): C, 70.39; H, 5.91; N, 12.07. Found C, 70.18; H, 5.82; N, 12.00%.

5.6.6. 7-Ethyl-3-phenylcarbamoyl-1-phenyl-5-(4-methylphenyl)-9-(4-methylphenylmethylene)-5H-6,7,8,9-tetrahydro[1,2,4]triazolo[4,3-a]pyrido[4,3-d] pyrimidine (**8f**)

Orange solid, yield (80%), mp 182–184 °C (Ethanol/Dioxane), IR (KBr, cm^{-1}) 3381(NH), 1645 (CO). MS *m/z* (%) 593 ($M^+ + 1$, 25), 592 (M^+, 100), 105 (48), 91 (50), 77 (85). Anal. Calcd. For $C_{38}H_{36}N_6O$

(592.75): C, 77.00; H, 6.12; N, 14.18. Found C, 76.92; H, 6.05; N, 14.03%.

*5.6.7. 7-Ethyl-3-phenylcarbamoyl-1-(4-methylphenyl)-5-(4-methylphenyl)-9-(4-methylphenylmethylene)-5H-6,7,8,9-tetrahydro[1,2,4]triazolo[4,3-a]pyrido[4,3-d] pyrimidine (**8g**)*

Yellow solid, yield (72%) mp 158 °C (Ethanol). ^{1}H NMR (DMSO-d_6) at $\delta = 0.95$ (t, $J = 7$ Hz, 3H, CH_3), 2.23, 2.26 (2s, 6H, $2CH_3$), 2.30 (s, 3H, CH_3), 2.33–3.42 (br, 6H, $3CH_2$), 6.31 (s, 1H, pyrimidine–H), 7.14–8.17 (m, 18H, ArH + benzylic–H), 10.61 (s, 1H, NH, exchangeable with D_2O). IR (KBr, cm^{-1}) 3398 (NH), 1653 (CO). MS *m*/*z* (%) 607 ($M^+ + 1$, 14), 606 (M^+, 35), 105 (28), 90 (75), 77 (100). Anal. Calcd. For $C_{39}H_{38}N_6O$ (606.78): C, 77.20; H, 6.31; N, 13.85. Found C, 76.99; H, 6.22; N, 13.66%.

*5.6.8. 7-Ethyl-3-phenylcarbamoyl-1-(4-chlorophenyl)-5-(4-methylphenyl)-9-(4-methylphenylmethylene)-5H-6,7,8,9-tetrahydro[1,2,4]triazolo[4,3-a]pyrido[4,3-d] pyrimidine (**8h**)*

Yellow solid, yield (71%) mp = 236 °C (Ethanol/Dioxane). ^{1}H NMR (DMSO-d_6) at $\delta = 0.85$ (t, $J = 7$ Hz, 3H, CH_3), 2.21(s, 3H, CH_3), 2.31 (s, 3H, CH_3), 2.50 (q, 2H, CH_2), 3.31–3.54 (4d, 4H, $2CH_2$), 6.21 (s, 1H, pyrimidine–H), 7.12–8.42 (m, 18H, ArH + benzylic–H), 10.66 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6) 12.17, 20.70, 20.74, 50.90, 52.40, 52.38, 59.28, 108.43, 120.35, 120.50, 122.95, 124.65, 126.57, 128.42, 128.81, 128.88, 129.09, 129.41, 131.74, 133.36, 134.21, 135.95, 136.71, 137.29, 137.96, 138.53, 139.26, 141.40, 143.60, 146.50, 153.88. IR (KBr, cm^{-1}) 3371 (NH), 1703 (CO). MS *m*/*z* (%) 628 ($M^+ + 1$, 21), 627 (M^+, 52), 626 (25), 564 (51), 625 (99), 152 (32), 119 (90), 105 (46), 91 (100). Anal. Calcd. For $C_{38}H_{35}ClN_6O$ (627.20): C, 72.77; H, 5.62; N, 13.40. Found C, 72.52; H, 5.43; N, 13.21%.

*5.7. Preparation of 7-ethyl-9-(4-methylphenylmethylene)-5-(4-methylphenyl)-2-thioxo-2,3,6,7,8,9-hexahydro-pyrimido[4,5-b][1,6] naphthyridine-(4H)-one (**12**)*

A mixture of compound **2** (3.3 g, 10 mmol) and 6-amino-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (1.43 g, 10 mmol) in glacial acetic acid (40 mL) was refluxed for 20 h. The reaction mixture was cooled and poured onto ice-cold water and the solid product was collected by filtration and crystallized from dioxane to give compound **12** as yellow solid. Yield (92%), mp 235 °C (Ethanol/Dioxane). ^{1}H NMR (DMSO-d_6) at $\delta = 0.80$ (t, $J = 7$ Hz, 3H, CH_3), 2.22 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.50 (q, 2H, CH_2), 3.13–3.54 (4d, 4H, $2CH_2$), 6.90–7.32 (m, 9H, ArH), 10.21(s, 1H, NH, exchangeable with D_2O), 12.0 (s, 1H, NH, exchangeable with D_2O). IR (KBr, cm^{-1}) 3420, 3356 (2NH), 1660 (CO). MS *m*/*z* (%) 454 (M^+, 23), 453 (15), 142 (15), 98 (46), 91 (100), 89 (39), 77(39). Anal. Calcd. For $C_{27}H_{26}N_4OS$ (454.60): C, 71.34; H, 5.76; N, 12.32. Found: C, 71.16; H, 5.60; N, 12.19%.

*5.8. Preparation of compound **15a**, **c**, **h***

To a mixture of equimolar amount of **12** and the appropriate hydrozonyl chloride **5a**,**c**,**h** (2.5 mmol of each) in ethanol (50 mL) was added TEA (0.35 mL, 2.5 mmol). The reaction mixture was refluxed till all hydrogen sulfide gas ceased to evolve (10 h monitored by TLC). The solvent was evaporated and the residue was treated with methanol. The solid that formed was filtered off and crystallized from the appropriate solvent to give compounds **15a**–**h**.

*5.8.1. 3-Acetyl-1-phenyl-6-(4-methylphenyl)-10-(4-methylphenylmethylene)-8-ethyl-7,9-dihydro[1,2,4]triazolo [4′,3′:1,2]pyrimido[4,5-b][1,6] naphthyridine-5-one (**15a**)*

Yellow solid, yield (82%) mp 240–242 °C (Ethanol). ^{13}C NMR (DMSO-d_6) 12.24, 18.12, 21.0, 21.18, 49.58, 50.57, 59.04, 110.24, 118.24, 124.50, 126.51, 126.45, 128.92, 129.04, 129.91, 130.27, 131.09, 132.11, 135.14, 136.0, 138.99, 139.25, 140.09, 141.52, 143.53, 146.23, 148.42, 154.33, 155.12, 158.70, 159.06, 162.0, 198.16. IR (KBr, cm^{-1}) 1710, 1682 (2CO), MS *m*/*z* (%) 581($M^+ + 1$, 10), 580 (M^+, 34), 422 (20), 91 (100), 105 (38), 77 (82). Anal. Calcd. For $C_{36}H_{32}N_6O_2$ (580.70): C, 74.46; H, 5.55; N, 14.47. Found: C, 74.25; H, 5.20; N, 14.65%.

*5.8.2. 3-Ethoxycarbonyl-1-phenyl-6-(4-methylphenyl)-10-(4-methylphenyl-methylene)-8-ethyl-7,9-dihydro[1,2,4]triazolo [4′,3′:1,2]pyrimido[4,5-b][1,6] naphthyridine-5-one (**15c**)*

Yellow solid, yield (73%) mp 224–226 °C (Ethanol). ^{1}H NMR (DMSO-d_6) at $\delta = 0.84$ (t, $J = 7$ Hz, 3H, CH_3), 1.25 (t, $J = 7$ Hz, 3H, CH_3), 2.35, 2.41 (2s, 6H, $2CH_3$), 2.49 (q, $J = 7$ Hz, 2H, CH_2), 3.25–3.72 (br, 4H, $2CH_2$), 4.38 (q, $J = 7$ Hz, 2H, CH_2), 7.09–8.20 (m, 14H, ArH + benzylic–H). IR (KBr, cm^{-1}) 1749, 1712 (2CO), MS *m*/*z* (%) 610 (M^+, 20), 608 (4), 607 (4), 482 (16), 435 (24), 119 (65), 105 (60), 91 (98), 77 (100). Anal. Calcd. For $C_{37}H_{34}N_6O_3$ (610.72): C, 72.77; H, 5.61; N, 13.76. Found C, 72.54; H, 5.48; N, 13.61%.

*5.8.3. 3-Phenylcarbamoyl-1-(4-chlorophenyl)-6-(4-methylphenyl)-10-(4-methylphenyl-methylene)-8-ethyl-7,9-dihydro[1,2,4]triazolo [4′,3′:1,2]pyrimido[4,5-b][1,6] naphthyridine-5-one (**15h**)*

Orange solid, yield (72%) mp = 262 °C (Dioxane). ^{1}H NMR (DMSO-d_6) at $\delta = 0.85$ (t, $J = 7$ Hz, 3H, CH_3), 2.23, 2.32 (2s, 6H, $2CH_3$), 2.36 (q, $J = 7$ Hz, 2H, CH_2), 2.80, 3.1, 3.52 (2d, 4H, $2CH_2$), 7.05–8.02 (m, 19H, ArH + benzylic–H), 11.94 (s, 1H, NH). IR (KBr, cm^{-1}) 3426 (NH), 1662 (CO); MS *m*/*z* (%) 694 ($M^+ + 2$, 2), 693 ($M^+ + 1$, 2), 692 (M^+, 4), 119 (100), 91 (74), 77 (17). Anal. Calcd. For $C_{41}H_{34}ClN_7O_2$ (692.23): C, 71.14; H, 4.95; N, 14.16. Found: C, 71.05; H, 4.71; N, 14.38%.

*5.9. Preparation of thiazolo[3′,2′:1,2]pyrimido[4,5-b][1,6] naphthyridine-3,5-dione (**17**)*

A mixture of compound **12**, bromoacetic acid (1.4 g, 0.01 mol), and fused sodium acetate (6 g) in glacial acetic acid (30 mL) and acetic anhydride (10 mL) was refluxed for 3 h, left to cool, then poured gradually with stirring onto cold water, the solid formed was filtered off, washed with water and crystallized from ethanol to give compound **17** as red solid, yield (60%), mp 226–228 °C (Ethanol). ^{1}H NMR (DMSO-d_6) at $\delta = 1.03$ (t, $J = 7$ Hz, 3H, CH_3), 2.24 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 2.56 (q, $J = 7$ Hz, 2H, CH_2), 3.19–3.38 (4d, 4H, $2CH_2$), 4.25 (q, 2H, CH_2), 7.02–7.24 (m, 9H, ArH + benzylic–H). MS *m*/*z* (%) 494 (M^+,6), 284 (12), 251 (21), 185 (13), 149 (30), 119 (51), 91 (40), 87 (89), 77 (11). Anal. Calcd. For $C_{29}H_{26}N_4O_2S$ (494.62): C, 70.42; H, 5.30; N, 11.33. Found: C, 70.62; H, 5.05; N, 11.11%.

*5.10. Preparation of 2-(3,4-dimethoxyphenylmethylene)-thiazolo [3′,2′:1,2]pyrimido[4,5-b][1,6]naphthyridine-3,5-dione (**18**)*

Method A: A mixture of compound **12** (4.5 g, 0.01 mol), bromoacetic acid (1.39 g, 0.01 mol) and fused sodium acetate (6 g) in glacial acetic acid (30 mL), acetic anhydride (10 mL) and aromatic aldehyde (1.66 g, 0.01 mol) was refluxed for 3 h, the reaction mixture was cooled and poured onto cold water, the solid formed was collected and crystallized from ethanol to give compound **18** as yellow solid, yield (65%), mp 238 °C (AcOH). IR (KBr, cm^{-1}) 1672, 1658 (CO), MS *m*/*z* (%) 642(M^+, 15), 476 (15), 152 (34), 119 (46), 117 (32), 110 (34), 105 (32), 98 (44), 91 (44), 78 (59), 77 (34), 57 (100). Anal. Calcd. For $C_{38}H_{34}N_4O_4S$ (642.78): C, 71.01; H, 5.33; N, 8.72. Found: C, 71.35; H, 5.09; N, 8.47%.

*5.11. Preparation of 7-ethyl-9-(4-methylphenylmethylene)-5-(4-methylphenyl)-2-hydrazino-2,3,6,7,8,9-hexahydro-pyrimido[4,5-b][1,6]naphthyridine-(4H)-one (**19**)*

A mixture of compound **12** (4.5 g, 0.01 mol) and hydrazine hydrate (85%, 5 mL) in ethanol (50 mL) was refluxed for 10 h. The reaction mixture was cooled and poured onto cold water and the solid formed was filtered off and crystallized from ethanol to give compound **19** as white solid, yield (60%), mp 275 °C (Ethanol). IR (KBr, cm^{-1}) 3416, 3328, 3240 (NH, NH_2), 1685 (CO), MS *m/z* (%) 453 ($M^+ + 1$, 47), 452 (M^+, 85), 364 (67), 318 (47), 222 (33), 198 (73), 154 (100), 140 (67), 89 (80), 81 (40), 78 (13). Anal. Calcd. For $C_{27}H_{28}N_6O$ (452.56): C, 71.66; H, 6.24; N, 18.57. Found: C, 71.54; H, 6.35; N, 18.29%.

References

[1] S.R. Kanth, G.V. Reddy, K.H. Kishore, P.S. Rao, B. Narsaiah, U.S.N. Murthy, Eur. J. Med. Chem. 41 (2006) 1011–1016.
[2] D.C.M. Chan, H. Fu, R.A. Forsch, S.F. Queener, A. Rosowsky, J. Med. Chem. 48 (2005) 4420–4431.
[3] R. Sharma, R.D. Goyal, L. Prakash, Phosphorus Sulfur Silicon Relat. Elem. 80 (1993) 23–29.
[4] Y.A. Issac, A.A. Aly, Z. Naturforsch., B: Chem. Sci. 58 (2003) 1227–1233.
[5] J. De Vry, E. Kuhl, P. Franken-Kunkel, G. Eckel, Eur. J. Pharmacol. 491 (2004) 137–148.
[6] S.M. Sondhi, V.K. Sharma, R.P. Verma, N. Singhal, R. Shukla, R. Raghubir, M.P. Dubey, Synthesis (1999) 878–884.
[7] D.L. Boyle, E.A. Kowaluk, M.F. Jarvis, C.H. Lee, S.S. Bhagwat, M. Williams, G.S. Firestein, J. Pharmacol. Exp. Ther. 296 (2001) 495–500.
[8] C.H. Lee, M. Jiang, M. Cowart, G. Gfesser, R. Perner, K.H. Kim, Y.G. Gu, M. Williams, M.F. Jarvis, E.A. Kowaluk, A.O. Stewart, S.S.S. Bhagwat, J. Med. Chem. 44 (2001) 2133–2138.
[9] H.N. Hafez, N.S. Abbas, A.B.A. El-Gazzar, Acta Pharm. 58 (2008) 359–378.
[10] R.K. Rawal, R. Tripathi, S.B. Katti, C. Pannecouquec, E. De Clercq, Bioorg. Med. Chem. 15 (2007) 3134–3142.
[11] H.N. Hafez, A.B.A. El-Gazzar, Bioorg. Med. Chem. Lett. 19 (2009) 4143–4147.
[12] R. Gupta, A.K. Gupta, S. Paul, P.L. Kachroo, Indian J. Chem. 37B (1998) 1211–1215.
[13] A.J. Elliott, P.D. Callaghan, M.S. Gibson, S.T. Nemeth, Can. J. Chem. 53 (1975) 1484–1488.
[14] All crystallographic data have been deposited at the Cambridge Crystallographic Data Center as supplementary publication no. CCDC 749447. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK.
[15] A.B.A. El-Gazzar, H.N. Hafeza, E.M.A. Yakoutb, J. Chinese Chem. Soc. 54 (2007) 1303–1312.
[16] A.B.A. El-Gazzar, N.A. Hassan, Molecules 5 (2000) 835–850.
[17] H.M. Hassneen, T.A. Abdallah, Molecules 8 (2003) 333–341.
[18] A.B.A. El-Gazzar, H.N. Hafez, Acta Chim. Solv. 55 (2008) 359–371.
[19] F.W. George, L. Johnson, J.D. Wilson, Endocrinology 125 (1989) 2434–2438.
[20] E. Disalle, D. Gindicen, G. Bricitico, G. Ornati, A. Panzer, J. Steroid Biochem. 46 (1993) 549–555.
[21] K.F. Austen, W.E. Brocklehurst, J. Exp. Med. 113 (1961) 521–539.
[22] N.F. Eweiss, A. Osman, J. Heterocycl. Chem. 17 (1980) 1713–1717.
[23] A.S. Shawali, A. Osman, Tetrahedron 27 (1971) 2517–2528.